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**A TISSUE CULTURE SOLUTION TO A FORESTRY PROBLEM — THE  
PROPAGATION OF A TETRAPLOID EUROPEAN ASPEN**

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**A Tissue Culture Solution to a Forestry Problem -  
The Propagation of a Tetraploid European Aspen**

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## ABSTRACT

A simple tissue culture method based on the production of multiple shoots from dormant buds is described. The application of the method to tetraploid European aspen illustrates that in vitro propagation can be an attractive alternative when conventional methods prove unsuccessful.

techniques became an attractive option to circumvent these difficulties in vegetatively propagating Ta-10. The following describes a general procedure that has been successfully used to propagate not only Ta-10 by tissue culture, but also other hardwood species as well.

#### MATERIALS AND METHODS

Dormant, lateral buds of Ta-10 were collected in January and February from the IPC arboretum near Greenville, WI. Buds were rinsed under cold water for 30 min and treated for 15 min with a 10% (v/v) solution of commercial bleach (Hilex). After three rinses with sterile water, the bud scales and outer leaves were aseptically removed, and the apical meristems with several layers of intact leaf primordia were again treated with 1% Hilex for 5 min. Following three rinses with sterile water, the explants were placed on woody plant medium (WPM; Lloyd and McCown, 1980) containing 0.05 mg/L naphthalene acetic acid and 1.0 mg/L benzyl adenine. The medium was adjusted to pH = 5.8 prior to autoclaving and solidified with 0.8% agar (Bacto; Difco). The cultures were incubated at 22°C and 3000 lux (cool-white fluorescent; 16/24 h photoperiod).

Every 2-3 days, the explants were transferred to renewed medium by sliding them to a different portion of the petri dish. Every two weeks, fresh medium was utilized. After 6-8 weeks "bud break" occurred, and shoots formed and multiplied. After 4 months, stable "shoot cultures" (see Fig. 1) could be maintained on the above medium without auxin. These cultures provided a continuous source of shoots suitable for rooting.

Root formation was accomplished in vitro as previously described (Wann and Einspahr, 1985). Briefly, 1/3 strength medium (macro and microelements) containing 0.1 mg/L indole butyric acid was used. Alternatively, shoots were

transferred directly from tissue culture to a mist bed for simultaneous rooting and hardening. Shoots or rooted plants were transferred to a soilless mixture containing equal parts sand, peat, and perlite and watered to saturation with 1 g/L benomyl (Benlate). Once established in soil, plants assumed growth rate and characteristics consistent with plants obtained from root sprouts (see Fig. 2).

## RESULTS AND DISCUSSION

Tetraploid European aspen could be readily propagated through tissue culture. Cultures were established that provided a year-round source of rootable shoots. Both bud break and root formation in vitro were nearly 100%. The procedure developed was not new but represented some of the best elements of several procedures for propagating aspen (Ahuja, 1984; Wann and Einspahr, 1985) and sweetgum (Sutter and Barker, 1985).

In the case of Ta-10, tissue culture provided a convenient means of vegetative propagation where several methods proved unsatisfactory. Situations similar to that encountered in Tc-10 can also arise in Leuci poplars where clones have been maintained by grafting but the outlet has been lost. In most hardwood the establishment of shoot cultures from lateral meristems is relatively simple and straightforward. For difficult to root species, the tissue culture systems can be considered as providing material at the requisite physiological stage (i.e., rejuvenated) for root formation. Aside from grafting, tissue culture in difficult to root species may provide an attractive method of vegetative propagation.

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Figure 1. Shoot culture of tetraploid European aspen (3X).



Figure 2. Tissue culture-derived tetraploid European aspen 10 weeks after transfer to potting medium.